Bacteriophage in the Sea

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Doklady Akad Nauk SSSR, 57: 833-836 (1947)

The question of the occurrence of bacteriophage in the sea has not yet been settled. Meanwhile, it is important for the development of ideas about conditions of life for microorganisms in marine environments. Bacteriophage belongs to a number of powerful biological factors determining the formation of microbic biosis, not only on the strength of its lytic action on bacteria, but also owing to its ability to cause hereditary changes and selection in bacterial populations.

d'Herelle tried to isolate bacteriophage from sea water. He found phage for enteric bacteria in the mouth of the Mekong and in the Mediterranian near Marcel, but he did not succeed in demonstrating bacteriophage in samples of water taken in the Indian Ocean at a great distance from shore. After him many investigators (3-5, 7) were engaged with search for bacteriophage for pseudomonas bacillus, dysentary bacillus, and the enteric-typhoid group in different seas. Such phages were found chiefly in harbors, in mouths of rivers, and in littoral zones; apparently these phages against pathogenic forms of microorganisms got into the sea with impure and sewer water. In the opinion of d'Herelle "everything that at any time comes into contact with animal defecation must contain bacteriophage." On the other hand, Davis (6) could not detect the presence of bacteriophage even in polluted water. According to an investigator of the Oceanographic Institute of the University of California, bacteriophage was detected in the sea water along the coast, but it did not once appear in samples of water beyond the littoral zone.

Thus [the literature lets one conclude that the occurrence of bacteriophage in the sea carries an accidental character; where discharge water containing fecal contamination happens to hit in the sea. By their composition these bacteriophages principally relate to a number of phages acting on enteric-typhoid group of bacteria. The present research was undertaken to get more information on this also.

In September 1946 a complicated oceanographic expedition for the study of the Black Sea, in which we took part, did a section from Yalta to Batumi. This section intersecting the central and eastern part of the Black Sea traversed parts of the sea most remote from the shore over maximum depth. At five stations along the profile 30 miles distant from each other, water samples were taken, and in four also soil samples. The depth of the first station was 2240 m, the second--2164 m, the third--2150 m, the fourth--2100 m, and the fifth was 1730 m.

Water samples to 250 m were withdrawn from depths 1, 25, 50, 75, 100, 125, 150, 175, 200, 225, and 250 m. Starting with 250 m, water

samples were taken from depths 500, 750, 1000, 1250, 1500, 1750, and 2000 m. Soil samples were obtained with an Ekman tube at the 2nd, 3rd, 4th, and 5th stations; only the uppermost layer of mud was investigated. Every sample of water and mud was seeded on fish-peptone agar, prepared from sea water, for isolation of bacteriological species, occurring at given depths. Differing colonies which grew on agar were streaked on agar slants and made up thus a collection of cultures of microorganisms characterizing the bacteriological population in different layers in the studied region of the Black Sea.

For absolute presence of bacteriophage in sea water and mud, all samples were divided into 4 groups. In the first group went water samples from depths 1, 25, 50, 75, and 100 m; in the second from depths 125, 150, 175, 200, 225, and 250 m; in the third from depths 500, 750, 1000, 1250, 1500, 1750, and 2000 m, taken from all 5 stations. The fourth group was made up of samples of the topmost layer of mud received from the 2nd, 3rd, 4th, and 5th stations. About 3 ml of each water sample and about 2 gm of mud made up the combined sample. The water samples of the first group combined together from 5 stations, in quantity about 75 ml, made up the pooled water sample from the layer from 1 to 100 m deep; the second group, in quantity 90 ml, made up the pooled sample from the layer from 125 to 250 m; the third group, in quantity 100 ml, from layer 500 to 2000 m. The pooled samples of mud represented the collection of the average sample of mud from 4 stations.

Detection of Bacteriophage: a) The Direct Method. The pooled water samples from layers 1 to 100 m, 125 to 250 m, 500 to 2000 m, and the mud samples were diluted with 100 ml of fish-peptone broth to prevent adsorption of phage on asbestos membranes and immediately filtered in Seitz apparatus.

b) Method of "additional sowing." This method, based on the principle of strengthening bacteriophage by lysis of homologous bacteria, was used in the following way. In test tubes with 3 ml of meat-peptone broth was added 1 ml of water of pooled sample from a given layer, or 1 ml suspension of pooled mud sample, and a loop of correlated broth culture, 18-20 hours old, isolated from water or mud. After keeping in an incubator at 25° for 18-22 hours, the contents of the test tubes from each layer were blended separately and filtered in Seitz apparatus.

c) Method of "passage." In the aim of further strengthening lytic effect of the filtrate, resulting by the method of additional sowing, they were passed on the technical bacterial cultures. The passage was produced by the above method, but only instead of water and suspension of mud with broth, to the homologous cultures 1 ml of filtrate was added.

The lytic effect of the filtrate obtained by the direct method, and the methods of additional sowing and passage was tested on cultures of bacteria, isolated from different depths, from where samples of water and mud were obtained. The general number of cultures tested

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equalled 143. For the tests broth cultures 18-20 hours old were used. Large drops of such broth cultures were streaked on the surface of meat-peptone agar. Filtrate or sterile broth (in control dish) was deposited by a droplet in the center of the streak. Parallelly another method was applied: 2 ml of filtrate was added to cooled, but not yet congealed, agar in the Petri dish. After solidifying the surface of this mixture was completely covered with a layer of broth culture. In all tests the meat-peptone broth and agar were prepared with added 1.8% NaCl. Test and control dishes were held in the incubator at 25° for 20-24 hours, and then the results were recorded.

Because of the limitation of space, only a small number of the actual materials are in Table 1. Selectively reduced data give representation of all variations in effect of the filtrate which was apparent in the process of the research.

By the direct method bacteriophage was detected in rare cases. Significantly more frequently it was apparent by the method of additional sowing. With the help of this method the presence of bacteriophage at great depths in the hydrogen sulfide zone of the Black Sea and in the mud was detected. The effect of the filtrate was demonstrated in the appearance of "taches vierges" on bacterial cultures, and in separate incidences marked zones of lysis were observed. Passage usually reinforced lytic strength of the filtrate; the number of taches vierges increased, or instead of many fine sterile spots--negative colonies-- lytic zones appeared, the rim of the culture acquired a pitted, lacy aspect. Bacteriophage often was observed not only on the agar medium, but also in inoculated broth cultures to which was added filtrate, following the methods of additional sowing or passage. It developed in appearance characteristic of agglutinated culture, or it was possible to judge it in the first 6 hours of observation by the absence of turbidity or of less turbidity in the broth in comparison with the control tube, which was inoculated with the same culture without added filtrate. In the range of marine forms of bacteria lysed by bacteriophage from water and mud of the Black Sea are included cocci, non-sporeforming motile and non-motile rods, and sporeforming kinds.

Crossing of determinations revealed that certain cultures isolated from specific depths could be lysed with filtrate obtained from very different layers of water, and also from mud. For example, bacteriophage against cultures Nos. 43, 111, and 94 were found not only in water layers 125-250 m, but also in layers 1-100 m, 500-2000 m, and in muds--a circumstance indicating wide diffusion of these phages in the water mass and also in the mud of the Black Sea.

The following facts also deserve attention. Streaks of cultures Nos. 66, 121, 55, and 87 in control dishes not exposed to the action of the filtrates were covered with great numbers of taches vierges. Cultures were clearly "contaminated" with bacteriophage or were

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Table 1. Bacteriophage in water and mud of the Black Sea

Pooled sample layer in meters	Culture number	Station & depth of culture isolation	Method of developing phage	Character of growth of culture: Drop of filtrate applied on a spot	On agar mixed with filtrate	Blank control
1-100	66	Sta. III 50 m	Direct	Taches vierges	Taches vierges	Taches vierges
	43	Sta. II 225 m	Additional sowing	t.v.	normal	normal
125-250	114	Sta. V 125 m	Direct	normal	light	normal
			Add. sowing	light	light	normal
			Passage (1)	normal	normal	normal
			Passage (2)	t.v.	lytic zone	normal
500-2000	102	Sta. IV 1250 m	Direct	normal	normal	normal
			Add. sowing	t.v.	light	t.v.
			Passage (1)	lytic zone	lytic zone	t.v.
	111	Sta. V 125 m	Add. sowing	lytic zone	t.v.	normal
	55	Sta. II 1750 m	Direct	t.v.	t.v.	t.v.
	1016	Sta. IV 1000 m	Direct	attenuated	light	normal
			Add. sowing	normal	normal	normal
			Passage (1)	normal	normal	normal
			Passage (2)	normal	normal	normal
Mud	143	Sta. V mud	Direct	normal	normal	normal
			Add. sowing	attenuated	light	normal
			Passage (1)	light	light	normal
			Passage (2)	t.v.	light	normal
			Passage (3)	attenuated	light	normal
			Passage (4)	lytic zone	t.v.	normal
	94	Sta. IV 175 m	Add. sowing	t.v.	t.v.	normal
	87	Sta. IV mud	Direct	t.v.	light	t.v.

Sta. = Station, t.v. = taches vierges (virgin spots), Add. sowing = additional sowing, light = light growth, attenuated = attenuated growth

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producing it, that is, they belonged to the number of so called lysogenic cultures. Such bacterial forms were often. They were isolated from different depths and from mud.

Bactericidal properties of sea water, which are reported in the literature (1, 8), it appears, are established not only with the presence of bacteriophage, but also with other factors. Illustrations, similar to them, such as were observed in experiments with culture No. 1016, when filtrate of sea water showed an inhibiting action of bacterial growth, were encountered relatively often. Contrary effect of bacteriophage in these cases indicates then, that filtrates obtained by the methods of "additional sowing" and "passage" did not cause any modification in the growth of cultures. Bacteriophages, developed in water and mud of the Black Sea, did not possess great lytic strength, if judged by the number and size of taches vierges. On the other hand, it is necessary to take into account with them, that cultures of bacteria from samples of water and mud, using the test-objective, possibly present populations composed significantly in their bulk of lytic resistant cells.

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December 1958

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